

<p style="text-align: center;">INTRODUCTION</p>	<p style="text-align: center;">Page viii</p>
<p style="text-align: center;">FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III</p>	<p style="text-align: center;">Issue No. 3</p>
	<p style="text-align: center;">Effective Date: 6-March-2006</p>
<p style="text-align: center;">INTRODUCTION</p> <p>An important development in the field of forensic science was the introduction of DNA typing systems for the analysis of biological samples. The RFLP-based typing system is highly informative, but requires greater than 50 ng of high molecular weight DNA and involves the use of multiple radioactive/chemiluminescent probes to obtain a result. An alternative approach to forensic DNA typing is based on the powerful polymerase chain reaction (PCR) technology, which does not require the use of radioactivity^{4,7}. Specific regions of the genome containing either sequence polymorphisms or length polymorphisms are amplified from minute amounts of DNA. Alleles which have been amplified and contain sequence differences may be typed using the "reverse dot blot technology"⁸. Amplified alleles containing length variations, such as short tandem repeats (STR's) and amplified fragment length polymorphisms (AMP-FLPs) may be typed by gel electrophoresis.</p> <p>STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length³. These abundant repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers which may be detected using the polymerase chain reaction. The alleles for these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using radioactive, silver stain, or fluorescence detection following electrophoresis. The PowerPlex® 16 BIO System provide a rapid, non-isotopic method which can be used to evaluate very small amounts of human DNA. STR typing is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500 bp long, much smaller than the material detected with AMP-FLP^{1,5} or VNTR^{2,6} analysis.</p> <p>The PowerPlex® 16 BIO System allows the co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin). The PowerPlex® 16 BIO System contains the loci Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818. In the system, one primer specific for Penta E, D18S51, D21S11, TH01, and D3S1358 is labeled with fluorescein (FL); one primer specific for FGA, TPOX, D8S1179, vWA, and Amelogenin is labeled with Rhodamine Red™ – X (RRX); and one primer is specific for Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 is labeled with 6-carboxy-4',5'-dichloro 2',7' – dimethoxyfluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube and analyzed in a single gel lane⁹.</p> <p>REFERENCES</p> <ol style="list-style-type: none"> 1. Allen, R., Budowle, B., Chakraborty, R., Giusti, A. and Eisenberg, A., (1991) "Analysis of the VNTR Locus AmpliFLP D1S80 by the PCR Followed by High-Resolution PAGE," Am. J. of Human Genetics, 48: 137-144. 2. Kasai, K., Nakamura, Y. and White, R., (1990) "Amplification of a Variable Number of Tandem Repeats (VNTR) Locus (pMCT118) by the Polymerase Chain Reaction (PCR) and its Application to Forensic Science," J. Forensic Sci., 35: 1196-1200. 3. Lygo, J.E., Johnson, P.E., Holdaway, D.J., Woodroffe, S., Whitaker, J.P., Clayton, T.M., Kimpton, C.P., and Gill, P., (1994) "The Validation of Short Tandem Repeat (STR) Loci for Use In Forensic Casework," Int. J. Leg. Med., 107: 77-89. 4. Mullis, K.B. and Faloona, F.A., (1987) Specific Synthesis of DNA <i>In Vitro</i> Via a Polymerase-Catalyzed Chain Reaction. <u>Methods in Enzymology</u>, ed. R.Wu., 155:335-350. 	

<p align="center">INTRODUCTION</p>	<p align="center">Page ix</p>
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<p>5. Nakamura, Y., Carlson, M., Krapcho, K. and White, R., (1988) "Isolation and Mapping of a Polymorphic DNA Sequence (pMCT118) on Chromosome 1p (D1S80)," Nucleic Acids Research, 16:9364.</p> <p>6. Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., et. al, (1987) "Variable Number of Tandem Repeat (VNTR) Markers for Human Gene Mapping," Science 235:1616-1622.</p> <p>7. Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N., (1985) "Enzymatic Amplification of -globulin genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia," Science 230:1350-1354.</p> <p>8. Saiki, R.K., Walsh, P.S., Levenson, C.H. and Erlich, H.A., (1989) "Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes," Proc. Natl. Acad. Sci. USA. 86:6230-6234.</p> <p>9. Promega PowerPlex® 16 BIO System Manual, Version 9/01</p> <p align="right">◆END</p>	